



Fig. S3. Multiplex immunofluorescence staining and automated image analysis. **a**, Schematic illustration of the automated image analysis of multiplex immunofluorescence staining. **b**, Left, representative multiplex immunofluorescence images for ACTA2 and CD31 in an NPC tumour sample. Right, quantitation of ACTA2⁺ and CD31⁺ cells as a percentage of all cells ($n = 6$). mean \pm s.e.m. **c**, Left, representative multiplex immunofluorescence images for CD4, CD8, CD20 and EPCAM in an NPC tumour sample. Right, quantitation of the percentages of CD4⁺, CD8⁺, and CD20⁺ cells within and outside of the area of tumour epithelial nests ($n = 6$). mean \pm s.e.m. **d**, Left, representative multiplex immunofluorescence images for IL3RA, CD57 and EPCAM in an NPC tumour sample. Right, quantitation of the percentage of IL3RA⁺ and CD57⁺ cells within and outside of the area of tumour epithelial nests ($n = 6$). mean \pm s.e.m. Scale bar: 50 μ m. * $P < 0.05$, and ** $P < 0.01$ (paired Student's t -test).